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# A Transcriptionally Active DNA-Binding Site for Human p53 Protein Complexes

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**Recent studies have demonstrated transcriptional activation domains within the tumor suppressor protein p53, while others have described specific DNA-binding sites for p53, implying that the protein may act as a transcriptional regulatory factor. We have used a reiterative selection procedure (CASTing: cyclic amplification and selection of targets) to identify new specific binding sites for p53, using nuclear extracts from normal human fibroblasts as the source of p53 protein. The preferred consensus is the palindrome GGACATGC CCGGGCATGTCC. In vitro-translated p53 binds to this sequence only when mixed with nuclear extracts, suggesting that p53 may bind DNA after posttranslational modification or as a complex with other protein partners. When placed upstream of a reporter construct, this sequence promotes p53-dependent transcription in transient transfection assays.**

The nuclear phosphoprotein p53 functions as a tumor suppressor, as demonstrated by its ability to inhibit transformation by viral and cellular oncogenes and by the high frequency of mutational inactivation of p53 in tumor cells (for review, see references 23, 37, and 40). It also cooperates with the retinoblastoma protein to regulate the M1 mechanism of in vitro cellular senescence (15, 32). The protein was initially characterized by its interaction with the products of oncogenic DNA tumor viruses such as simian virus 40 (SV40), human papillomavirus, and adenovirus, each of which expresses a protein that complexes specifically with the p53 protein. The binding of p53 to the large T antigen of SV40 inhibits several of the activities of the T antigen, including binding to the viral origin of replication (5, 13), interaction with DNA polymerase α (39), and ATP-dependent helicase activity (13). Mutants of p53 which can act in a *trans*-dominant fashion to inactivate the wild-type p53 protein usually fail to bind to large T antigen, suggesting that p53 may interact with a functional homolog(s) of T antigen in normal cells.

Several lines of evidence suggest that p53 acts in part as a DNA-binding protein. p53 binds nonspecifically to DNA-cellulose (18, 21, 35), and purified recombinant wild-type p53 has been shown to bind to sequences at the SV40 DNA origin of replication (2), as well as to specific sites found in human genomic DNA sequences suspected of controlling normal cellular DNA replication (19). Some mutant versions of p53 fail to bind DNA in either a specific or a nonspecific fashion (2, 19, 35). DNA protection patterns on the SV40 chromosome induced by p53 implicate the Sp1-type GC boxes (GGGCGG) as binding sites (2), while protection patterns of a p53-binding human genomic DNA clone suggest that 5-bp repeats of TGCCT are required for binding (19). In addition, fusion proteins composed of the DNA-binding domain of GAL4 linked to p53 have demonstrated the presence of a strong transcriptional activation domain within the p53 molecule, suggesting that the protein may participate in transcriptional promoting complexes (11, 25–27).

We describe here the use of native p53 complexes to determine the DNA-binding specificity of p53. In a procedure which we have termed CASTing (cyclic amplification and selection of targets) (42), nuclear extracts that contain the DNA-binding protein of interest are mixed with an excess of double-stranded oligonucleotide containing a central core of complete degeneracy flanked by polymerase chain reaction (PCR) priming sequences. After the DNA-protein complexes are purified by using magnetic beads coated with monoclonal antibody, the DNA is released and amplified by PCR. Following several CASTing cycles, the DNA is ligated into an appropriate vector and the DNA sequences of individual clones are determined. Related approaches that use repetitive enrichment of sites present in degenerate oligonucleotides have been developed independently in several laboratories (3, 7, 10, 34, 38). A strong palindromic DNA-binding site for p53 was observed after six CASTing cycles, and this sequence confers p53-dependent activation when placed upstream of a reporter gene in transient transfection assays.

## MATERIALS AND METHODS

**CASTing for the p53 consensus DNA-binding site.** CASTing was performed essentially as described previously (42). The target oligonucleotide contains flanking PCR primer sites which bracket a central core of 35 degenerate bases (Fig. 1A). A 5-μg sample of this mixture was converted to double-stranded DNA by annealing an excess of a primer complementary to the 3′ flanking site and then performing one primer extension reaction with *Taq* DNA polymerase. Nuclear extracts were prepared from proliferating normal human lung diploid fibroblasts (IMR90, ATCC CCL186, passage 29) as described elsewhere (22). Anti-p53 monoclonal antibody beads were prepared by incubating anti-mouse immunoglobulin G-coated magnetic beads (Dynal Inc., Great Neck, N.Y.) with culture supernatant from PAb421 hybridoma cells. The first cycle of CASTing included 10 μg

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**A**

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   XhoI        EcoRI        XbaI        SalI
CGCTCGAGGGATCCGAATTC-N35-TCTAGAAAGCTTGTCGACGC
        BamHI                    HindIII
```

**B**

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  CTAAGGCCCCCATCCG GGACA TGCCC GGGCA TGTT
SGAAAAATAGATGGTGGG GAACA TGCCC GGGCA TGtct
              GAAG GGACA TGCCC GGGCA TGTCG GAAACGATA
AGCGAACTGTAAGTGCGG GGGCA TGCCC GGGCA TGtct
       CGCAAACGATA GAACA TGCCC GGGCA TGTCC AGGG
                AC AAACA TGCCC GGGCA TGTTC CTGCTGTGGACCT
GCGGGGACATTAGGGACG GGACA TGCCC GGGCA TGtct
    ACGAAATCCTTCAT GGACA TGCCC GGGCA TGTCG
                AC AAACA TGCCC GGGCA TGCCC CATGTGA
                   GGGCA TGCCC GGGCA TGTTC TTGGAATAACAACCT
GACCCACACCTAATGTCA AGACA TGCCC GGGCA TGtct
                   GGACA TGCCC GGGCA TGTCT GTAGTTCCCTCTAC
                   GGACA TGCCC GGGCA TGCCC AAAATAAGACCATTA

 CGGTACTCTGCATGATA GGACA TGTCC GGACA TGTtc
GCCCACTACCAATGAGCG GGACA TGTCC GGACA TGtct

              CTGCCC AAACA TGTCC GGGCA TGTCC GTGACGTGG
TGAATATTGAGGCTTCAC GGACA TGTCC GGGCA TGtct
```

**C**

```
GGACATGCCCGGGCATGTCC
CCTGTACGGGCCCGTACAGG
```

FIG. 1. PAb421-selected DNA sequences following six CASTing cycles. (A) Sequence of the degenerate oligonucleotide. PCR primers which correspond to the cloning sites that flank the degenerate bases were used to amplify the selected DNA between each cycle. (B) DNA sequences of individual clones following six rounds of CASTing (42). Seventeen clones contained conserved palindromic elements, which are aligned and highlighted in boldface type. The three groupings define sequences with slightly different arrangements of the consensus half-sites (see below). Bases from the *Xba*I site adjacent to the degenerate core are shown in lowercase. Nuclear extracts were prepared from proliferating normal human diploid fibroblasts (IMR90, passage 29) as described elsewhere (22). Six additional clones (not shown) contained sequences apparently unrelated to those shown above. These have not been examined by EMSA to determine whether they contain specific binding sites and may represent random sequences still present after six cycles of CASTing. (C) Configuration of potential half-sites in the extended p53 consensus binding site. Each consensus site can be defined as containing four half-sites: two of TGCCC and two of TGTCC. The sequences of individual clones in panel B suggest that several different arrangements of these half-sites can support p53 binding.

of double-stranded oligonucleotide and 4 μl of IMR90 nuclear extract in a total volume of 20 μl of buffer (100 mM NaCl, 20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid] [pH 7.5], 1.5 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg of pepstatin per ml, 10 μg of leupeptin per ml, 0.1% Triton X-100, 20% glycerol). Following a 20-min incubation at room temperature, 10 μl of PAb421 anti-p53 antibody-coated magnetic beads was added, and the mixture was then agitated at room temperature on a low-speed shaking platform for 1 h. The magnetic beads were retrieved from solution with a magnet and then washed four times with 0.5 ml of isotonic saline containing 0.1% Nonidet P-40 and 0.1 mg of bovine serum albumin per ml. The DNA bound to the beads was then released by adding 100 μl of PCR reaction mixture containing 500 μM each of the two primers which flank the degenerate oligonucleotide and 125 μM each of deoxyribonucleotide in *Taq* reaction buffer (Promega, Madison, Wis.) and then heating at 95°C for 5 min. *Taq* DNA polymerase (1 μl) was then added, followed by 10 to 20 cycles of PCR (94°C, 60 s; 55°C, 60 s; 72°C, 120 s). Amplification products were analyzed by agarose gel electrophoresis (2% agarose, 1× TBE (Tris-borate-EDTA), and 10 μl of the amplified material (typically containing 30 to 50 ng of DNA) was used to initiate a second round of CASTing. The second and subsequent rounds of CASTing included 10 μg of sonicated salmon sperm DNA as a nonspecific competitor during the initial incubation of nuclear extract with oligonucleotide. Following six cycles of CASTing, the amplified DNA was digested with *Bam*HI and *Hin*dIII and then cloned into pBluescript II (Stratagene, La Jolla, Calif.), and the sequences of individual inserts were determined by the dideoxy method by using double-stranded plasmid.

**EMSA.** Mouse C2C12 myoblasts or human diploid fibroblasts (IMR90 at PDL 28) were transfected with 10 μg of an expression vector for human wild-type p53 (pC53-SN) or the parental vector pCMV-Neo-Bam (pCMV) (1). Electroporations were performed on $1.5 \times 10^7$ cells in a volume of 0.5 ml with a GenePulser apparatus (Bio-Rad, Richmond, Calif.) set at 250 V/960 μF. The total amount of DNA in each transfection was increased to 60 μg with sonicated salmon sperm DNA. Nuclear extracts were prepared 48 h posttransfection as described elsewhere (22). An electrophoretic mobility shift assay (EMSA) was performed on approximately 1 ng of end-labeled double-stranded oligonucleotide by using 3 μl of nuclear extract (10 μg of protein) and 0.5 μg of sonicated salmon sperm DNA as a nonspecific competitor in a total volume of 12 μl of the buffer described for the initial step of the CASTing procedure. After a 20-min incubation at room temperature, the products were separated on native polyacrylamide gels (4%, 1× Tris-borate-EDTA).

**In vitro translations.** The small *Bam*HI fragment of plasmid pC53-SN containing the p53 coding sequence was cloned into plasmid pBluescript II $KS^+$, and the resulting plasmid was linearized by digestion with *Eco*RI. Capped transcripts were prepared by using T7 RNA polymerase (Promega) and then translated in reticulocyte lysates as recommended by the supplier (Promega). The translation products were assayed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and 3 μl of these was assayed directly for DNA binding to end-labelled double-stranded oligonucleotide in the presence of 0.5 μg of sonicated salmon sperm DNA as nonspecific competitor. In mixing experiments, 3 μl of translation products was added to 3 μl of nuclear extracts prepared from IMR90 fibroblasts and left on ice for 30 min prior to initiating the EMSA.

**Luciferase reporter gene assays.** pGUP.PA.8 was obtained from R. S. Williams (41a). It contains a basal promoter element including the TATAA element from the human *hsp70* gene upstream of the firefly luciferase coding sequence (9). Double-stranded oligonucleotides encoding the p53 consensus binding site (p53CON), fragment A (containing nucleotides 106 to 138 of fragment A from human genomic clone 772 $C_{BE}$ [19]), or both were first cloned and sequenced in pBluescript II (Stratagene) and then released by restriction digestion and cloned into the *Sma*I-*Bam*HI cloning site located 5′ to the basal promoter of pGUP.PA.8.

H1299 cells, a human non-small cell lung cancer line that contains a homozygous deletion of the p53 gene (24a), were transfected by electroporation as described above. Each transfection included 10 μg of reporter plasmid, 10 μg of pCMV-lacZ, 10 μg of either pC53-SN or pCMV, and 30 μg of sonicated salmon sperm DNA. Luciferase activity was assessed (9) 24 h following transfection and normalized for differences in transfection efficiency as determined by a

spectrophotometric β-galactosidase assay (29). The results are reported as the means of duplicate experiments.

**Nucleotide sequence accession number.** The EMBL accession number for the DNA-binding motif recognized by p53-containing complexes described in this report is X63571 HSDBMP53.

## RESULTS AND DISCUSSION

We have determined DNA-binding sites for p53-containing nuclear complexes from normal diploid fibroblasts. Our previous study on the DNA-binding specificity of the muscle regulatory factor myogenin indicated that the preferred site for purified recombinant protein homomers differed significantly from that of the protein isolated from nuclear extracts; in the latter case, the protein is complexed to its natural protein partners (12, 42). Normal diploid fibroblasts contain low but detectable amounts of p53, and we chose to use crude nuclear extracts from these cells as a source of wild-type p53 protein to reflect a native configuration of the protein.

Figure 1B presents a list of DNA sequences cloned after six cycles of CASTing with the anti-p53 monoclonal antibody PAb421 and nuclear extracts from IMR90 human lung fibroblasts. Of the 23 clones initially sequenced, 17 contained a clearly recognizable palindromic element. Examination of these sequences indicates that they represent a tandem arrangement of two shorter imperfect palindromes, each containing the half-sites TGCCC and TGTCC (Fig. 1C). Each clone therefore contains a string of potential half-sites which can apparently be arranged in a variety of combinations to support p53 binding.

EMSAs were performed to demonstrate binding of p53 to the CASTing consensus sequence, p53CON. We were initially unable to demonstrate specific gel shift activity using nuclear extracts from IMR90 fibroblasts. Since the levels of p53 protein in these extracts are extremely low, we increased p53 expression by transfecting the mouse myoblast cell line C2C12 (Fig. 2) or IMR90 fibroblasts (Fig. 3) with a cytomegalovirus promoter-driven wild-type human p53 expression vector, pC53-SN, and analyzed the resulting nuclear extracts. As shown in Fig. 2, transfection with the parental pCMV vector alone did not result in a specific shifted band, although addition of PAb421 antibody to this assay produced a faint shifted band (compare lanes 2 and 3). Transfection with the p53 expression vector induced a strong band (Fig. 2, lane 4) which was double shifted by PAb421 and double-shifted and/or abolished by a different anti-p53 monoclonal antibody, PAb1801 (lanes 5 and 6, respectively). Antibody alone did not interact with the probe in the absence of nuclear extract (Fig. 2, lane 1), indicating the presence of p53 in the double-shifted complexes present in lanes 5 and 6. The p53-specific band was subject to competition with an excess of unlabeled self oligonucleotide (Fig. 2, lanes 7 to 9) but not with two unrelated oligonucleotides (NF1 and MEF1 [Fig. 2, lanes 10 and 11]) nor with an oligonucleotide (fragment A [lane 12]) previously described as containing a specific p53-binding site (19). Fragment A includes nucleotides 106 to 138 of human genomic clone 772 $C_{BE}$, which contains four copies of a TGCCT motif. (It should be noted that the lower nonspecific band in Fig. 2 and 3 was subject to competition with both the NF1 and fragment A oligonucleotides. The DNA half-site of the NF1 protein family, TGGC, is similar to the TGCCC and TGTCC half-sites present in p53CON and to the TGCCT half-sites present in fragment A,

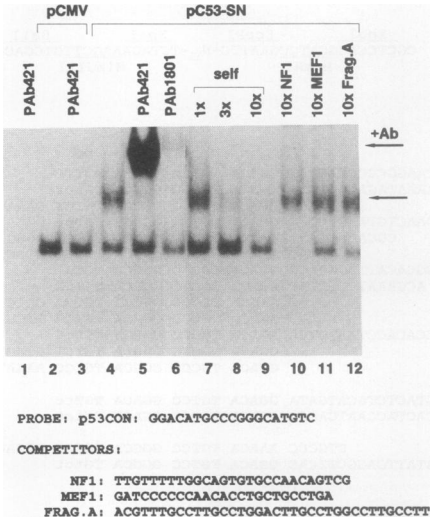


FIG. 2. EMSA of the selected consensus binding site demonstrates specific DNA binding. Mouse C2C12 myoblasts were transfected with an expression vector for human wild-type p53 (pC53-SN) or with the parental vector pCMV. p53-specific binding is demonstrated by the formation of tertiary complexes with anti-p53 antibodies (lanes 5 and 6) and by competition with self (lanes 7 to 9) but not with other oligonucleotides (lanes 10 to 12). A 1-μl volume of anti-p53 monoclonal antibody PAb421 (10-fold-concentrated hybridoma culture supernatant) or PAb1801 (100 μg of Ab-2 per ml; Oncogene Science, Manhasset, N.Y.) was added as indicated. Nonlabeled double-stranded oligonucleotides used as competitors were included in molar excess compared with labeled probe. NF1, 20-bp oligonucleotide containing a consensus binding site for NF1 protein ($TGG_{N6-7}CCA$); MEF1, 26-bp sequence to the MEF1-binding site of the mouse MCK enhancer (6); fragment A (FRAG. A), 32-bp sequence from fragment A (nucleotides 106 to 138) of clone $772C_{BE}$ (19), previously shown to bind to purified recombinant wild-type p53. Lower arrow, position of the p53-specific band; upper arrow, position of antibody double-shifted band.

and it is possible that this nonspecific band is due to binding by members of the NF1 family.)

Both antibodies appear to displace entirely the p53-induced band, but the intensity of the PAb421 double-shifted band was considerably greater than the original p53-induced band, while that of the PAb1801 band was considerably less. Different epitopes are recognized by antibodies PAb421 (C terminus of p53) and PAb1801 (N terminus of p53) (43). It is likely that the binding of PAb421 to its epitope either releases the protein from a complex in which the protein has low affinity for this DNA or stabilizes the protein in a conformation which has increased affinity for DNA, while binding to the PAb1801 epitope may do the reverse. This effect is similar to that recently observed with an antibody to the estrogen receptor on binding to an estrogen response element (17). The faint double-shifted PAb421 band seen in extracts from nontransfected or from control pCMV-transfected extracts (Fig. 2, lane 3) would result from the effect of PAb421 on the endogenous mouse p53 in these cells. In support of this conclusion, PAb421 double-shifted bands are totally absent in nuclear extracts from human cell lines (MDA-MB-157, ATCC HTB24, or H358 [4]) which lack detectable amounts of p53 protein (data not shown).

Some of the essential features of this binding site were examined by testing the ability of mutant sequences to compete with the labeled CASTing consensus p53-binding

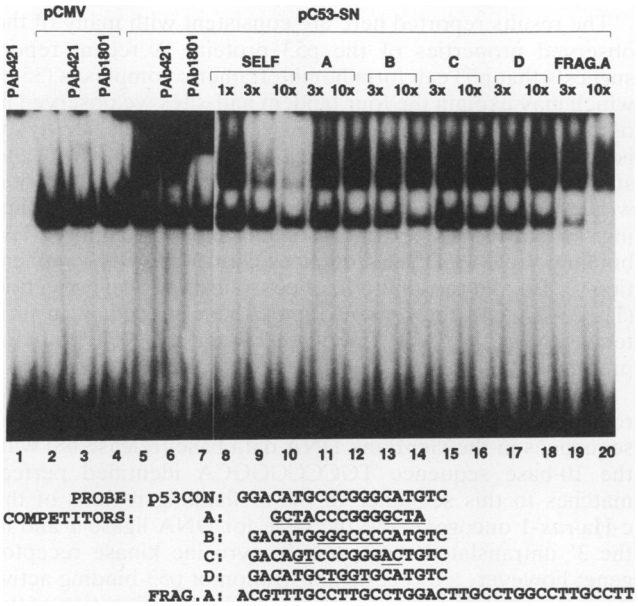


FIG. 3. Competition for p53 binding by variants of the consensus sequence. Normal human lung fibroblasts (IMR90 and PDL 29) were transfected with plasmids pCMV or pC53-SN. Nuclear extracts were prepared 40 h posttransfection and were mixed with approximately 1 ng of labeled p53CON oligonucleotide by using 0.5 μg of sonicated salmon sperm DNA as nonspecific competitor. Anti-p53 antibodies (PAb421 or PAb1801) or nonlabeled competitor oligonucleotides (as molar excess of labeled probe) were included prior to the addition of nuclear extract as indicated. Neither the mutant sequences A to D nor fragment A (Frag. A) competed for binding of the p53-specific complex. Substitutions from the consensus sequence in competitors A to D are underlined.

site. As seen in Fig. 3, EMSA analysis of diploid IMR90 fibroblasts transfected with the p53 expression vector yielded induced and double-shifted bands similar to those seen with mouse C2C12 myoblasts (compare Fig. 2, lanes 1 to 9 with Fig. 3, lanes 1 to 10). Neither mutations in which the central CCCGGG block was preserved while the flanking sequence was changed (competitor A; Fig. 3, lanes 11 and 12) nor a mutation containing an inverted version of this block in which the flanking sequences were maintained (competitor B; Fig. 3, lanes 13 and 14) could compete effectively for binding. Inversion of the TG and CA dinucleotides distal to this central block also negated competition (competitor C; Fig. 3, lanes 15 and 16), as did substitution of this block with a nonpalindromic sequence (competitor D; Fig. 3, lanes 17 and 18).

Figure 4 compares the ability of p53 to recognize p53CON versus the probe fragment A. The complex binding to fragment A was double shifted by monoclonal antibody PAb421 when assayed with nuclear extracts from normal fibroblasts transfected with a wild-type human p53 expression vector, although the intensity of the PAb421-shifted band was considerably reduced in comparison to the same shifted band using the core sequence p53CON (Fig. 4; compare lanes 4 and 8). Fragment A competed poorly with p53CON for p53 binding at the DNA concentrations tested (Fig. 3, lanes 19 and 20), indicating that fragment A either binds p53 with lower affinity than the CASTing consensus or binds p53 in some alternate conformation or complex.

Previous reports have demonstrated nonspecific interaction of p53 with DNA when in vitro-translated (18) or

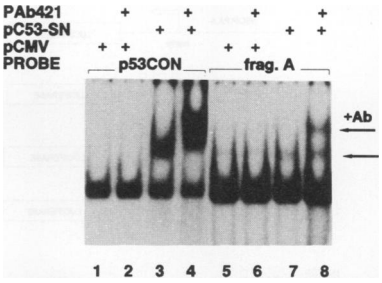


FIG. 4. EMSA of the consensus sequence versus fragment A (frag. A). End-labeled p53CON or fragment A oligonucleotides were assayed for binding to nuclear extracts as described in the legend to Fig. 3 in the presence or absence of antibody PAb421. Lower arrow, position of the p53-shifted band; upper arrow, position of the PAb421 double-shifted band.

bacterially expressed protein (33) binding to total genomic DNA was used or sequence-specific interactions when purified baculovirus-expressed protein (2, 19) was used. To determine whether p53 binds to p53CON directly as nonmodified protein, we prepared in vitro-translated wild-type human p53 and assayed the reaction products by EMSA. The background pattern of bands present in nonprogrammed reticulocyte lysates (RL in Fig. 5 [lanes 2 and 3]) was unchanged in comparison with that of in vitro-translated p53 protein (53 in Fig. 5 [lanes 4 and 5]), either in the absence or in the presence of antibody PAb421. We reasoned that if p53 requires additional modification or required oligomerization with another protein, mixing the translated protein with nuclear extracts might reconstitute an active complex. The faint band due to the endogenous p53 detected by antibody PAb421 (present in mixtures of nuclear extract and nonprogrammed reticulocyte lysate; Fig. 5, lane 7) became prominent when in vitro-translated p53 was mixed with the same extract (Fig. 5, lane 9). Similar results were obtained when nuclear extracts from the p53 null cell line H358 were used; however, the activity ascribed to the endogenous p53 was absent.

These findings suggest that in order to bind to this specific sequence, p53 must either form complexes with additional proteins present in nuclear extracts or be posttranslationally modified by the extract. The palindromic nature of the

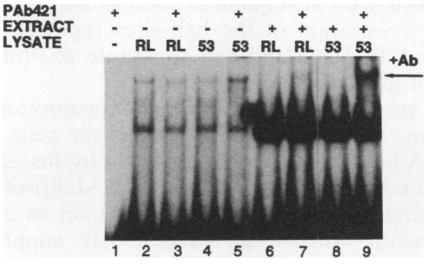


FIG. 5. EMSA of in vitro-translated p53 demonstrates that p53 protein alone is insufficient for binding to the consensus. Binding of in vitro-translated human wild-type p53 (53; lanes 4, 5, 8, and 9) to p53CON oligonucleotide was assayed in comparison with that of nonprogrammed rabbit reticulocyte lysate (RL; lanes 2, 3, 6, and 7) either directly (without extract [−]) or following a 30-min preincubation with nuclear extracts prepared from IMR90 cells (with extract [+]) (22). Monoclonal antibody PAb421 was included as indicated. Position of the band double shifted by the antibody is indicated by an arrow.

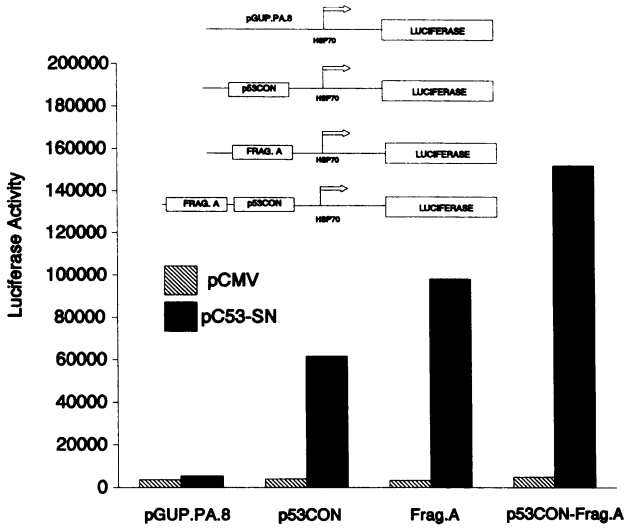


FIG. 6. Luciferase reporter gene assays of p53 DNA-binding sequences. Oligonucleotides encoding the p53 consensus binding sequence p53CON, fragment A (Frag. A), or both were cloned 5′ to the basal hsp70 promoter element of the luciferase reporter plasmid pGUP.PA.8. Plasmids were cotransfected with the p53 expression vector pC53-SN or the parental vector pCMV, and the level of luciferase activity was determined 24 h posttransfection. Activities were normalized by measuring β-galactosidase activity from a cotransfected lacZ expression vector, pCMV-lacZ.

consensus sequence is a feature which frequently indicates protein dimer-binding sites (16), suggesting that p53 binds across the consensus site either as a pair of modified homodimers or possibly as paired heterodimers with other nuclear proteins. The ability of p53 to form homo- or hetero-oligomers (8, 20, 31, 36) supports such a conclusion. In a separate experiment, digestion of the CASTing consensus oligonucleotide by *Sma*I (which produces a blunt-end cut in the middle of CCCGGG sequences) into half-molecules severely disrupted the ability of the consensus to act as self-competitor (data not shown). Although one-half of this molecule would still retain an inverted repeat unit (Fig. 1C), the reduction in binding affinity would suggest that paired dimer-binding sites provide some degree of cooperativity in the binding of protein to DNA. We suspect that the repeated TGCCT motif seen in sequences such as that of fragment A represents a variation of the half-sites reported here (i.e., TGCCC and TGTCC) and can constitute p53-binding sites by virtue of their physical proximity.

The p53 protein contains a potent transcriptional activation domain, as demonstrated by reporter gene studies in which DNA-binding activity is provided by fusion of p53 to the DNA-binding domain of the yeast GAL4 protein (11, 25 to 27). To determine whether p53 could act as a transcriptional activator without an exogenously supplied DNA-binding activity, we inserted p53CON, fragment A, or both adjacent to the basal hsp70 promoter of the luciferase reporter plasmid pGUP.PA.8. When assayed in the p53 null cell line H1299, single copies of either the p53 consensus binding site or fragment A were capable of increasing the activity of this promoter approximately 15- and 20-fold, respectively, when cotransfected with a wild-type p53 expression vector (Fig. 6). A tandem linkage of p53CON with fragment A provided a 30-fold activation, indicating an additive rather than a synergistic effect.

The results reported here are consistent with many of the observed properties of the p53 protein. A recent report suggests that p53 can form homotetrameric complexes (35a), which may explain the four tandem half-sites we observed in our CASTing consensus-binding sequence. The ability of isolated half-sites, such as those seen in fragment A, to support both p53 binding and transcriptional activation would suggest that natural binding sites may require groupings of half-sites only in reasonably close proximity. The binding of p53 to DNA sequences controlling DNA replication (2, 19) and the ability to effect transcriptional activation (11, 25–27, and the present report) have precedent in proteins such as the SV40 T antigen (28) and the NF1 family of proteins (24).

We have observed paired half-sites in the noncoding regions of many human genes. A screen of the human sequences in the GenBank DNA data base (release 68) with the 10-base sequence TGCCCGGGCA identified perfect matches to this sequence in the 5′ flanking regions of the c-Ha-*ras*-1 oncogene and the gene for DNA ligase 1 and in the 3′ untranslated region of the tyrosine kinase receptor gene; however, a direct demonstration of p53-binding activity in these genes has not yet been made. Identification of the actual genes targeted for regulation by p53 will be of great interest. Since overexpression of p53 has been shown to both activate and repress the transcription of different genes (14, 30, 41), p53 is likely to be part of a regulatory cascade that controls cellular replication and thus may be an important component of normal cellular division and senescence. Knowledge of normal p53 functions should eventually lead to a clearer understanding of the effects of p53 mutations in oncogenesis.

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